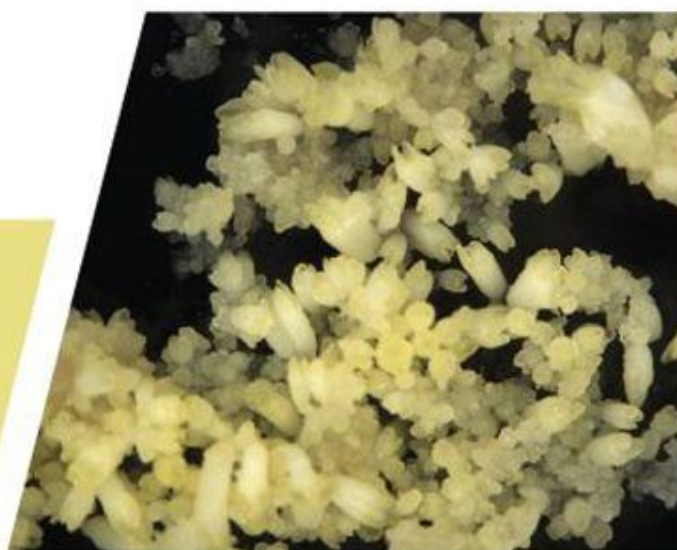


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Teak

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Abstract

Teak (*Tectona grandis* Linn. f.) is one of the most prized high value timber species. Industrial teak plantations have in recent times rapidly expanded due to shrinking supplies followed by a total ban on harvesting teak from natural stands. The clonal option offers an attractive means of mass producing superior quality planting stock of this species focusing on phenotypic criteria and wood properties. Protocols for large scale propagation by rooted cuttings and *in vitro* microcuttings of mature selected teak trees have been developed to meet the shortage of planting material. The respective pros and cons of these two techniques are reviewed in this chapter, emphasizing the comparative advantages of the efficient tissue culture procedure for meeting increasing requirements of fast growing and premium quality teak planting material in the wet tropical regions worldwide.

Keywords: Axillary budding; Clonal propagation; International dispatch; Meristems; Micropropagation, Rejuvenation; Planting stock improvement.

1. Global status of teak

Tectona grandis Linn. f., commonly known as teak, is a large and long-lived arborescent tree belonging to the *Lamiaceae* family and is native to India, Laos, Myanmar (ex-Burma) and Thailand (Tewari 1992). It remains one of the most prized high value timber due to the outstanding properties of its wood, with special mention for durability and aesthetic features (FAO 2009). This attractiveness has spurred the introduction of the species for timber production in several tropical countries of Asia, starting with Indonesia some 4 to 6 centuries

ago(Siswamartana 2000; Verhaegen et al. 2010), then Africa and Latin America (Ball et al. 2000). The list of countries that have embarked on industrial teak plantations has rapidly expanded, recently brought about by private investors eager to meet the demand for high grade timber in the wake of declining supplies from natural stands (FAO 2009; Kollert and Cherubini 2012).

Current expectations are to produce teak wood from commercial plantations with much shorter rotations than the longer ones from natural stands, thus giving these establishments a high comparative advantage to become a main source of supply. As demand for plantation-grown teak increases, the private sector has increasingly become involved in commercial plantations. All this has become possible through the judicious use of selected superior, fast growing genetic planting material that produces a high volume of quality wood in the shortest possible time frame (Ugalde 2013).

2. Rationale for propagating teak vegetatively

Sexual propagation through seeds remains for teak, as for most species, the easier and the more natural way to produce new plants, with each seedling being genetically different from another. This creates genetic diversity and is useful for genetic improvement through sound breeding activities. Propagating teak by seeds has been traditionally practiced for centuries, with the possibility of storing the seedlings in the form of “stumps” until the suitable planting season (Kaosa-ard 1986). However, mass production of superior teak planting stock by seeds is impeded by several limitations such as insufficient quantities of fruits produced, low germination rates and a positive correlation between flowering age and forking height (White 1991; Kaosa-ard et al. 1998, Callister 2013). The sooner teak trees produce flowers, the shorter their clear bole length, and hence, the lower their market value. Seed-derived teak trees, even from the same progeny, also show substantial variability for economically important traits that are assumed to be mainly under non-additive control (Kjaer et al. 2000; Callister and Collins 2008; Chaix et al. 2011; Monteuis et al. 2011).

In 1996, Kjaer and Foster wrote that it will take at least 50 to 70 years before genetically improved teak plantations established from seeds can be harvested while uncertainties associated with the resulting practical genetic gain will remain. Kjaer et al. (2000) further stressed that such breeding strategies will remain heavily penalized by low seed productivity, with average yield of 50 kg per ha from age 15 according to Wellendorf and Kaosa-ard (1988), and overall poor germination rates. The large demand for improved seeds on the one hand and limited productivity of the clonal seed orchards on the other, makes seed procurement a difficult business. At present, a large part of the seed is collected

from the more easily accessible seed sources, e.g. road sides and urban areas, consisting mostly of short branchy trees that are fruiting prematurely and abundantly. This is very likely the main reason for the poor quality of most seed-derived teak planting stock. These aspects have been discussed by White and Gavinlertvatana (1999) who concluded that the “seedling route is outdated and actually represents a deterrent to increased productivity in teak plantations”, and as such, to commercial teak plantation investment. According to these authors, the magnitude of the real genetic gain associated with the seedling route has yet to be clearly defined, and the basic question of whether all the efforts invested in it during the past decades are worthwhile, has remained.

In contrast, asexual or vegetative propagation consists in duplicating, theoretically in unlimited numbers, selected genotypes while preserving through mitotic divisions their original genetic make-up, and consequently all their individual characteristics including the economically important traits poorly inherited through seeds. Further, vegetative propagation can be applied to any individual that does not produce fertile seeds, either because it has not yet entered the mature stage or as a result of unfavorable environmental conditions.

Similarly as for other tree species (Zobel and Talbert 1984), applying vegetative propagation to teak can be useful for research as well as for operational activities. Research aspects encompass:

- (i) Clonal tests, in order to compare and identify superior clones for operational planting (Goh et al. 2013a, Monteuis and Goh 2015);
- (ii) Genotype X environment interactions for selecting the clones to be deployed according to their adaptability to planting site conditions (Goh and Monteuis 2012);
- (iii) Genetic parameter estimates, including broad sense heritabilities, genetic correlations between traits and the magnitude of “C effects” (Callister and Collins 2008, Goh et al 2013a); and
- (iv) *Ex-situ* conservation of particular genotypes and gene complexes for germplasm enrichment and further use in other environments.

The operational usefulness of vegetative propagation for teak can be for:

- (i) Establishing clonal seed orchards, being aware of the limitation of low productivity, but also of the benefits, i.e., the improved and thereby superior genetic quality of the seedlings produced (Chaix et al. 2011, Monteuis et al. 2011, Goh et al. 2013b); and
- (ii) Mass producing rooted cuttings for cutting forestry that can be implemented in the form of monoclonal blocks of various sizes or of clones planted in mixtures (Monteuis and Ugalde Arias 2013).

In such a situation, mixing the clones at the plantation level reduces the impact of genotypes that may not be well adapted to the site, contrary to large

monoclonal blocks that are more uniform. Nonetheless, in spite of the deployment option, mass propagating the different genotypes separately, regardless of the added constraints of it being more laborious and time-consuming, permits the number of representatives of each clone to be known for a better control of the genetic composition of the tree populations in the field. This will prevent the risk of having genotypes with the higher multiplication and rooting capacity, but not necessarily the best field performers, supplanting others, thereby leading to the reduction in the genetic base to ultimately only a single clone.

3. Vegetative propagation of teak by *ex vitro* methods

3.1 Grafting and budding

Teak grafting, especially by budding with success rates of nearly 100% in Thailand (Kaosa-ard 1998), has in the past been the most widely practised vegetative propagation technique for establishing clonal seed orchards or *ex-situ* gene banks (Singh and Beniwal 1993). It is a low-cost technology applicable to any teak genotype regardless of its age, providing that the usual basic requisites are met (Hartmann et al. 1997).

However, grafting or budding gives rise to genetically “composite” plants made up of the selected genotype of the grafted scion and of the unselected genotype of the rootstock. This is liable to produce shoots faster than the selected material that was grafted and from which the rootstock shoots cannot be visually distinguished as each looks very much alike to the other, hence frequently resulting in “illegitimates” (Bagchi et al. 1991). The occurrence of such “illegitimates”, which may affect a significant proportion of the clonal seed orchards, is likely to depreciate the genetic quality of the seeds produced by illegitimate “mothers” and also by the surrounding “legitimates” fertilized by genetically polluted pollen (Bagchi et al. 1991, Tilakaratna and Dayananda 1994). The consequences can be even more serious when such “illegitimates” are used as stock plants for mass clonal propagation by rooted cuttings for operational planting. Seed producers or stock plants clonally produced on their own roots, for instance, by cuttings or microcuttings prevent such risks: they either grow or die, and illegitimates do not arise. Another aspect associated with the production of clones by grafting or budding is the possible influence of the seed-derived rootstock on the performance of the grafted scion, such as reduced vigor and a branchy architectural development. Lastly, the quality of the connection between the stock and the grafted scion can also become a risk as an additional source of within-clone phenotypic variability.

3.2 Layering

Successful propagation of teak by layering was reported for shoots sprouting from the stump of a 33-year-old felled tree (Lahiri 1985). Mound layering from 5 year-old felled teak trees gave success rates ranging from 45% to 81% depending on the time of the experiment (Monteuuis et al. 1995). However, this technique requires the felling of the donor tree. If it is not cloned, there is a risk of losing the superior genotype, and thus this technique is not practically adaptable to large scale operations.

3.3 Propagation by rooted cuttings and minicuttings

An efficient technique for rooting cuttings from teaks of various ages, including individuals which had entered the flowering stage a long time ago and could therefore be classified as physiologically mature (Hackett 1985; Wareing 1987), was developed during 1992-1994 in Sabah, East Malaysia (Monteuuis 1995; Monteuuis et al. 1995). Subsequently, the successful transfer of this procedure to various tropical countries has confirmed its efficiency for clonally mass propagating by rooted cuttings a wide range of teak Plus trees regardless of their age while preserving their characteristics. Setting sections or “sticks” cut from low branches of the selected tree under shaded and mist system facilities stimulates the production of elongating shoots that can subsequently be used as cuttings for rooting the selected mature genotypes (mobilization phase). Thereafter, the first generation of vegetative copies from the original ortet can be obtained. This method is practically preferred, it being more conservative, to the use of coppice shoots arising from the stump of the selected tree that had been felled (Palanisamy and Subramanian 2001; Singh et al. 2006; Husen and Pal 2007). As in layering, there is indeed always a risk that the felled Plus tree does not produce sprouts from the stump and ultimately dies. Generally represented by a sole individual, which is the case for most seed-derived candidate Plus trees, this technique may result in the loss of the genotype. The “stick” method, further used with success by Surendran and Muralidharan (2007) and thereafter by Akram and Aftab (2009) for cloning 40 to 50 year-old teak Plus trees, has also proven to be more practical and efficient than (serial) grafting or budding onto younger rootstock (Husen and Pal 2003; Shirin et al. 2005).

The few first rooted cuttings obtained from this mobilization phase were then managed intensively as stock plants before embarking on a serial propagation or “cascade process”. The capacity for adventitious rooting of the plant material increases gradually with the number of successive generations of cascade. Average rooting rates of 70% can be obtained after three cycles of serial propagation (Monteuuis 1995; Monteuuis et al 1995). This apparently is the minimal level of rooting responsiveness required for embarking on large scale production of teak rooted cuttings under cost-efficient conditions.

3.4 Advantages and limitations

Propagation by rooted cuttings of selected teak genotypes of various ages has thus proven to be feasible, with sufficiently high success rates to be compatible with cost-effective large-scale production. For instance, KVTC in Tanzania has routinely used this cloning procedure for producing up to 250,000 rooted cuttings/per annum from mature selected teak genotypes (Hans Lemm, personal communication). Such good results depend, however, on a few basic requirements, such as:

- (i) Suitable nursery facilities (Monteuuis et al. 1995), consisting mainly of adjacent shaded areas: one for maintaining the container-grown stock plants under intensive management, especially with regard to watering, feeding, and hedging/pinching operations (Hartmann et al. 1997) and another nearby area equipped with a reliable mist system (Hartmann et al. 1997) for rooting the cuttings, and then facilitating the weaning and hardening processes prior to field planting;
- (ii) Efficient mobilisation and rejuvenation techniques as detailed by Monteuuis et al. (1995) for physiologically rejuvenating the mature selected genotypes in order to improve their adventitious rooting ability and suitable shoot-producing capacity for successful rooting; and
- (iii) Adapted stock plant management for stimulating the production of shoots with the highest potential for adventitious rooting. Such shoots are characterised by distinctive morphological traits as described previously (Monteuuis 1995, Monteuuis et al. 1995). This must be considered the determining factor for ensuring good rooting rates. It requires special care, skills and techniques, particularly, where attention and observation are concerned and which are often underemined in practice.

More recently, different Latin American countries, in particular, Costa Rica, Brazil, Guatemala, have developed mass clonal production of teak trees using young vegetative minicuttings rooted under aeroponics-fog system conditions (Monteuuis and Ugalde Arias 2013). This system, which has proven to be quite efficient and attractive, requires suitable stock plant management and sophisticated greenhouse facilities equipped with a reliable and high quality fog system. Similar to more traditional methods of propagation by rooted cuttings, stock plants must have a high capacity for adventitious rooting, requiring prior physiological rejuvenation of the genotypes selected from mature teak trees. Nonetheless, the full production cost of these aeroponics-derived minicuttings has to be taken into consideration and might be a limitation under a commercial production set up.

The main limitations compared to tissue culture procedures are:

- an overall lower production efficiency and the effect of climatic changes;

- the bigger space requirement of the facilities;
- the competence of human resources required to collect and maintain the stockplants in an adequate condition for adventitious rooting, particularly if obtained from mature selected genotypes; and
- the limitations, if not impossibility, to export the produced rooted minicuttings to overseas countries owing to stringent phytosanitary requirements of each importing country.

4. *In vitro* micropropagation by axillary budding

Teak can also be vegetatively propagated by tissue culture (Gupta et al. 1980; Mascarenhas and Muralidharan 1993; Sunitibala Devi et al. 1994; Suhaendi 1998). In Sabah, East Malaysia (Monteuuis et al. 1998; Goh and Monteuuis 2001), Thailand (Kaosa-ard et al. 1987; Gavinlertvatana 1998), and in Brazil (Monteuuis and Ugalde Arias 2013), large scale micropropagation activities have been successfully developed for domestic as well as for international markets under the impetus of private companies (<http://proteca.com.br>; <http://www.ysgbiotech.com>; http://www.semseo.co.uk/doc/index.cfm?id_doc=575).

Although somatic embryogenesis, particularly of unicellular origin, may be useful for genetic engineering, micropropagation by axillary budding has been preferred to micropropagation by adventitious budding and somatic embryogenesis for large scale *in vitro* production of teak. This is due to higher culture sustainability and genotypic fidelity compared with *de novo* procedures. It is estimated that to date, several millions tissue-cultured teak plantlets have been micropropagated using this axillary budding technology. However, based on the increasing number of plantations that have been established by using clonal planting materials over the past 10 years, particularly in Latin America, the actual scale could very likely be in the double digit million figures. The total amount is difficult to determine accurately as information on sales by the supplier companies generally remain confidential per agreement between supplier and buyer.

The *in vitro* technology described in the subsequent paragraphs was developed by the YSG Biotech Sdn Bhd, Yayasan Sabah Group, where it has been applied with great satisfaction for almost 2 decades from seeds and uppermost, from field-selected Plus trees (Monteuuis et al. 1998; Goh and Monteuuis 2001).

4.1 From seeds

In vitro culture conditions can be very useful for rapidly increasing the number of individuals obtained from seeds of presumably high genetic value but available only in limited number and with low germination capacity (Akram and Aftab 2007). This may be the case of provenances or progenies derived, for

instance, from controlled pollination, or from clonal seed orchards (Yasodha et al. 2005). The beneficial effects of tissue culture is to improve the germination capacity as well as to vegetatively propagate the newly *in vitro* germinated genotypes (Monteuuis et al. 1998). These are mostly propagated as a mixture as they are too young to be reliably selected for individual clonal propagation. This *in vitro* “bulk propagation” can be applied for various lengths of time, depending on needs. However, during the course of the successive propagation cycles, the risk of narrowing the original genetic base owing to the potentially higher multiplication rates of certain genotypes over others could become a problem and should not be underestimated.

Once developed to the right height, the *in vitro* germinated seedlings can be cut into microcuttings to be clonally micropropagated depending on the objectives in mind, for instance, for among- and within-clone variability assessment. In practice, however, this option remains far more cumbersome than “bulk propagation” and is not practically warranted since any teak tree, regardless of its age, can be successfully mass micropropagated.

4.2 From field-selected plus trees

The field-selected phenotypes to be micropropagated *in vitro* can be of any age, including *in situ* individuals as well as nursery stock plants, provided vegetative buds can be collected. About 1cm-long mononodal (single node), and terminal portions from vegetative shoots, preferably actively growing, are routinely used for initiating the *in vitro* cultures with one explant per test tube in order to limit the loss from possible microorganism contaminations. Thereafter, during the subsequent stabilization and production phases, flasks containing 8 to 10 microcuttings each are then used for the mass production. Records from several years of experimentation with different-aged field-grown genotypes established that, subject to the disinfection procedure and depending on the manipulator, 20 to 30% of these primary explants could give rise to contamination-free and responsive *in vitro* cultures (Monteuuis et al. 1998; Goh and Monteuuis 2001). Overall, it takes 6 to 8 months to achieve, through serial subcultures of explants collected from mature selected donor trees, the level of physiological rejuvenation required for large scale production.

Shoot apical meristems or SAMs are big enough (overall size of 0.1 mm) in teak to be used as primary explants, which is not the case for those of most other tree species. The decussate leaf pattern of the species facilitates their excision from the apical buds of the growing donor shoots and skilled people can routinely inoculate 30 to 40 teak SAMs per hour onto proper *in vitro* culture media (Monteuuis et al 1998). In addition to higher success rates and efficiency than

nodal explants for initiating contamination-free cultures (70 vs 20-30%, respectively), especially as far as endogenous contaminants are concerned, SAMs used as primary explants are more efficient for achieving physiological rejuvenation from mature selected genotypes (Monteuuis 1989; Monteuuis and Goh 2015).

The tissue culture protocols used were designed to be as simple as possible in order to be easily applicable, even by non-tissue culture specialists, and to reduce the constraints of large-scale applications. Cost-efficiency and high productivity are in this respect essential. Regardless of the origin of the initial plant material (*in vitro* germinated seedlings or outdoors individuals), or of its age and of the kind of primary explant used (nodal or terminal segments or 0.2mm-long SAMs), the established technology allows for the mass micropropagation under *in vitro* conditions of any genotype, either in bulk or individually, through axillary-produced microshoots with an exponential multiplication rate of 3 to 4 cuttings at every 6 week-long sub-culture. Finally, 50 to 60% of the microcuttings can root spontaneously in the sole multiplication-elongation culture medium during the production phase. Further, the rooting-acclimatisation phase was advantageously achieved in nursery conditions under a mist system with more than 90% success on average in the absence of any application of rooting substance. This confirms that for physiologically rejuvenated material, application of “growth hormones” is not necessary and that the environmental conditions at the acclimatization site are the most important factors at this stage, consistently with previous observations (Bonal and Monteuuis 1997; Monteuuis et al. 1998).

Mortality during the subsequent steps of cultivation in the nursery, before the plants are sufficiently developed to be field-planted, is negligible. To date, millions of microcuttings have been produced by applying this technique, and have developed into vigorous and true-to-type vegetative offspring (Goh and Monteuuis 2012, Goh et al 2013a; Monteuuis and Goh 2015).

4.3 Advantages and limitations

For teak as for any other species that can be tissue cultured (Bonga and von Aderkas 1992), the assets of micropropagation compared to conventional propagation methods, i.e. by rooted cuttings in a nursery, are:

- Year-round production regardless of the local climatic conditions.
- Requirement of only a small space area even for huge numbers of plants produced in flasks.
- Suitably managed stockplants, with nursery facilities and associated competent staff required for their proper maintenance, are not necessary.
- Production and packing of contamination-free plants that meet phytosanitary

requirements for exportation to foreign countries.

- Higher efficiency for achieving the physiological rejuvenation needed for clonally mass propagating mature selected trees true-to-type.

In addition, the comparative advantages of the protocol developed for teak in our case lie in:

- the utilization of a unique elongation-multiplication medium for the production phase, thus reducing the use of resources in relation to time and costs (labor, culture medium, overhead expenses),
- that rooting is easily undertaken at a much cheaper cost under nursery conditions.
- the possibility to use SAMs as primary explants with the above-mentioned benefits, and
- the simplicity of procedure does not require specialized and highly paid staff.

Conversely, and potentially, the few limitations are:

- higher proportion of contaminations and longer delays for physiologically rejuvenating the mature selected plant material when nodal or terminal shoot portions are used as primary explants. SAMs have the advantage of overcoming these problems,
- limited multiplication rates when using the more natural way of multiplication by axillary budding. However, this process offers sustainability, simplicity and efficiency as well as true-to-typeness of the tissue-culture plants produced.

5. Conclusion

From our viewpoint, the basic reasons for the success of micropropagation in teak are:

- the universal reputation of teak as one of the most prized high value timber species,
- the increasing international demand for fast growing planting material that will produce premium quality teak wood in order to achieve higher returns on investment in short time frames,
- the availability of superior clones, greatly preferable to seedlings for meeting investors' goals, which depends on the access to outstanding trees that can be mass propagated true-to-type using a very efficient cloning technique, and
- the optimization of the overall process from initiation to multiplication to exportation, and ultimately, the successful *ex vitro* acclimatization of the microcuttings at the buyers' facility.

These latter conditions were met in Sabah, East Malaysia by the Yayasan Sabah Group Biotech where efficient *in vitro* and nursery protocols for mass cloning true-to-type teak Plus trees selected from highly diverse base and breeding populations (Goh and Monteuis 2009) were developed. Comparative economic

analyses have clearly shown within this context that for the production of more than 100,000 rooted cuttings per year, the tissue culture procedures developed are more efficient ((Monteuuis et al 1998; Monteuuis 2000; Goh and Monteuuis, 2001). This is mainly due to the fact that although the *in vitro* option must take into account the establishment of a laboratory, it does not require stock plants which need to be intensively managed by competent people as is the case for the nursery option (Monteuuis et al. 1995). The investments required at the nursery level increase in far greater proportions per production target than for micropropagation.

Due to the simplicity of the *in vitro* procedure developed, it can be easily handled by committed low level local workers who are paid less than in countries with a higher standard of living. This makes the production cost of the plantlets cheap and assures a reasonable selling price backed by the renowned quality of the planting material produced when the process is shifted from seedling-derived to clonal planting materials, which is expected to rise in volume in the future (Ugalde Arias 2013). Market prospects can be further improved with the possibility of sending tissue-cultured plants off to different destinations, at various distances, as a result of phytosanitary immunity, contrary to rooted cuttings (Goh and Monteuuis, 2001). To date, several millions of teak vitroplants have been produced by YSG Biotech and sent to different countries all around the world, including Australia, South America, Africa and within South East Asia. The possibility for such international dispatches lies in having a well-coordinated system in place, from the production of plants per order received to the endorsement by the local quarantine authority in both countries and finally, to the efficient communication among suppliers, buyers and freight agents involved. All this bring about the minimization of untoward risks and the timely arrival of the consignment at the buyer's country within 3 to 5 days, bearing utmostly in mind, the limited shelf life of these live plants.

Unlike many forest tree species, teak plants from cuttings and microcuttings develop true-to-type, in the absence of any phenotypic abnormalities such as undesirable plagiotropic growth patterns that are noted to affect (micro) cuttings of many forest species – the so-called “C effects” (Frampton and Foster 1993). Growth rates are impressive in the first few years, with 4 m of annual increment under evenly distributed high rainfalls in the absence of a long dry season (Goh and Monteuuis 2012, Goh et al. 2013a, Monteuuis and Goh 2015). In addition to this impressive growth, it is noteworthy that the cloned plants developed under such conditions have long clear boles devoid of forks and with very few lateral branches. All these positive features attest to the validity of mass selection based on phenotypic criteria brought about by the efficiency of the developed clonal techniques for teak.

Today, with the ownership of two high quality teak progeny-provenance

plots, comprising of up to 42 families from a broad genetic background, YSG Biotech continues to improve their clonal materials by providing plants that are adapted to different site conditions in tropical and sub-tropical teak-growing regions. Using the developed techniques, the possibility to supply superior quality clonal materials will undoubtedly sustain the establishment of large-scale industrial plantations with more predictable lucrative returns in the near future. Clonal forestry for a high value timber species such as teak and no doubt, other economically-important species, is here for the long haul based on the successful application of vegetative propagation techniques through tissue culture and nursery cuttings.

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